Department of Pesticide Regulation

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M E M O R A N D U M

TO: John Donahue, Chief **HSM-94001**
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FROM: Tom Thongsinthusak, Staff Toxicologist **[original signed by Tom Thongsinthusak]**
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DATE: April 7, 1994

SUBJECT: DETERMINATION OF DERMAL ABSORPTION OF PESTICIDES IN ANIMALS

I. Background Information:

Most dermal absorption studies of pesticides submitted in support of pesticide regulation are conducted using experimental animals. Rats are commonly used animals that comprise about 70% of the studies. The approximate percentage of the studies using other animals and humans are: humans 15%, monkeys 9%, pigs 4%, and rabbits 2%. Dermal absorption studies in animals typically follow the general guidelines suggested by Zendzian (1987, 1989), except for humans and monkeys where the methods follow the procedures given by Feldmann and Maibach (1974) or Wester and Maibach (1985). Scientists at DPR routinely review the study protocols and provide comments and suggestions upon request by the registrants. The variables for a dermal absorption study that yield the most efficient data, including administered doses, exposure times, sacrifice times are presented in Table 1 and also in HS-1612 (Thongsinthusak *et al*., 1993). Study design is determined in part by the nature of the units that the final result will be expressed in. For risk assessment purposes, DPR determines percent absorbed dosage from a dermal absorption study.

Studies conducted for a maximum of 24 hours from the time of dosing almost invariably have a large percentage of the dose associated with the skin following washing. Bound skin residues (i.e., residues recovered in the treated skin sites) are normally included in the dermal absorption values if bioavailability of these residues cannot be determined. This is not the case where asymptotic extrapolation of percent of dose excreted in excreta (urine and feces) can be performed. This extrapolation method has been used successfully for the determination of dermal absorption of several pesticides, including propargite (Thongsinthusak *et al*., 1989), cyromazine (Thongsinthusak, 1991), tralomethrin (Thongsinthusak, 1993a), tribufos (Thongsinthusak, 1993b), and azinphos-methyl (Thongsinthusak, 1994).

II. Desirable Attributes of Study Design Conductive to Asymptotic Extrapolation:

The dose administered site is typically washed with aqueous surfactant at 10 hours post exposure. New protective appliances are attached and the animals are returned to metabolic cages. Samples of urine and feces are collected daily until the end of the study, that is 96 hours or longer. These samples are analyzed separately and the results are expressed as percent of dose. Table 2 shows the tabulated dose excreted in urine and feces of animals treated with tralomethrin at 3 ug/cm^2.

Table 1. Recommended doses, exposure times, and sacrifice times for a dermal absorption study in experimental animals.

Dose (ug/cm^2)	Exposure time (Hours)[a]	Sacrifice time (hours)[b]
1-6	4, 10	4, 10, 24, 96 or longer
10-25	4, 10	4, 10, 24, 96 or longer
50-100	4, 10	4, 10, 24, 96 or longer

[a] Four-hour exposure is for a sacrifice time four hours after initiation of exposure, whereas, ten-hour exposure is for sacrifice times at 10, 24 hours or longer after initiation of exposure.

[b] For the sacrifice time of 24 hours or longer, the treated skin site is washed or rinsed with soap solution at 10 hours, e.g. 2% Ivory liquid soap or equivalent in distilled water. Urine and feces samples are collected daily until the final sacrifice time. These samples are analyzed and the results reported as % dose.

Table 2. Percent of dose excreted in rat urine and feces after being treated with tralomethrin at 3 ug/cm^2.

Time (hours)	Percent of dose (mean)			
	Urine (U)	Feces (F)	U+F	Cumulative (U+F)
24	0.56	0.10	0.66	0.66
48	0.61	0.36	0.97	1.63
72	0.54	0.25	0.79	2.42
96	0.33	0.20	0.53	2.95
120	0.28	0.15	0.43	3.38

III. Asymptotic Extrapolation of Percent Dose Excreted in Urine and Feces:

a) Software package:

Systat and Sygraph (A registered Trade Mark of Systat, Inc.). Other software packages (e.g., NONLIN, SAS) with similar capability may be used for the same purpose.

b) Model for asymptotic plots:

Exponential saturation model with lag time is used for this purpose. This exponential model is generally applicable to describe excretion kinetics. In this case, the dermal absorption study involves absorption, distribution, and excretion of the chemical. The goal of this method is to estimate dermal absorption by extrapolating the dose recovered in excreta to a time at which the cumulative excretion curve has plateaud. The curve describing the cumulative quantity of radiolabel excreted as a function of time resembles a hyperbola with an upper maxima of 100 percent.

Model: RECOV = MAX*(1-EXP(-RATE*(Time+LAG)))

Or $Y = A[1-e^{-B(X+C)}]$

Statistical concerns:

1) The Nonlin program may not converge on parameter estimates if the starting values are far off from the true values.

2) Also, local minima may allow convergence on erroneous parameter estimates. Plotting the data with the best fit line will reveal obviously bad parameter estimates.

3) Even with high quality parameter estimates, we must remember that the asymptote estimate is an extrapolation **beyond** the range of actual data, although if excreta are collected long enough to reach plateau the results are obvious.

4) The asymptote estimate is a point estimate with an associated uncertainty. Therefore, it may be best to include the 95% confidence intervals or a ±SE with the estimate.

IV. Dermal absorption of tralomethrin at 3 ug/cm^2:

a) A dermal absorption value is calculated as follows:
% Dermal absorption = Sum of % dose at asymptote (or MAX) plus percent dose recovered in carcass, blood, and cage washes.

b) Percent dermal absorption is adjusted for incomplete recovery from a mass balance calculation.

Or % Dermal absorption (adjusted) = (% Dermal absorption x 100)÷(Total dose recovery)

V. Conclusions:

An ideal method to resolve the question of whether bound skin residues should be included in a dermal absorption estimate is to extend the study until there is no radiocarbon in the excreta. This practice may take weeks for some pesticides and it is not practical in a typical dermal absorption study. A study using an appropriate protocol should yield data that can be used to estimate maximum excretion of dose. Therefore, bound skin residues at treated skin sites can be disregarded. For our purpose, an exponential saturation model with lag time is considered appropriate. The dermal absorption estimate is the sum of percent dose at asymptote and percent of dose in carcass, blood, and cage washes. Without adequate data for this type of asymptotic extrapolation, bound skin residues are considered as part of a dermal absorption value.

This method of estimating dermal absorption has several advantages. It uses all the data and does not rely on a single point estimate to derive dermal absorption. The output of the curve fitting yields statistics which help define the level of uncertainty in the absorption estimate. Finally the method is well grounded in classical principles of pharmacokinetics and offers a rationale for estimation of dermal absorption consistent with risk assessment as currently practiced.

VI. References:

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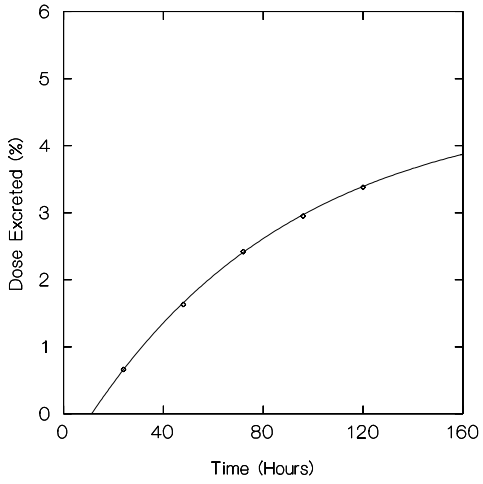
Figure 1. Asymptotic plot of cumulative excretion of dose of tralomethrin in urine and feces at different time intervals for 3 ug/cm^2 dose.

$$Y = 4.6549*(1-EXP(-0.0119*(X-11.2803)))$$



Statistics:

>MODEL RECOV=Max*(1-EXP(-Rate*(Time+Lag)))
>ESTIMATE/ ITER= 25 START= 4,.011,.1 SIMPLEX SCALE

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ITERATION	LOSS	PARAMETER VALUES		
0	.4423074D+00	.4000D+01	.1100D-01	.1000D+00
1	.2924814D+00	.4873D+01	.8706D-02	.1538D+00
2	.1058626D+00	.6004D+01	.6795D-02	.1650D+00
3	.7113534D-01	.6571D+01	.6012D-02	.1847D+00
4	.6071989D-01	.7566D+01	.5059D-02	-.4267D-02
5	.5877466D-01	.8229D+01	.4550D-02	-.1789D+00
6	.5832284D-01	.8565D+01	.4349D-02	-.4303D+00
7	.5710997D-01	.8394D+01	.4531D-02	-.1194D+01
8	.4308329D-01	.7452D+01	.5442D-02	-.4603D+01
9	.3753377D-01	.6969D+01	.6101D-02	-.6778D+01
10	.3108027D-01	.6429D+01	.6682D-02	-.5969D+01
11	.2390916D-01	.6266D+01	.7173D-02	-.7450D+01
12	.1822321D-01	.5625D+01	.8358D-02	-.8481D+01
13	.1037538D-01	.5273D+01	.9430D-02	-.8637D+01
14	.3036110D-02	.4697D+01	.1154D-01	-.1062D+02
15	.1298963D-02	.4684D+01	.1174D-01	-.1104D+02
16	.1027378D-02	.4642D+01	.1199D-01	-.1136D+02
17	.1013248D-02	.4654D+01	.1192D-01	-.1129D+02
18	.1013029D-02	.4655D+01	.1191D-01	-.1128D+02
19	.1013022D-02	.4655D+01	.1191D-01	-.1128D+02
20	.1013022D-02	.4655D+01	.1191D-01	-.1128D+02

DEPENDENT VARIABLE IS RECOV

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE
REGRESSION	29.0748	3	9.6916
RESIDUAL	0.0010	2	0.0005
TOTAL	29.0758	5	
CORRECTED	4.6995	4	

RAW R-SQUARED (1-RESIDUAL/TOTAL) = 1.0000
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) = 0.9998
STANDARD ERRORS OF PARAMETERS ARE RESCALED

PARAMETER	ESTIMATE	A.S.E.	LOWER <95%>	UPPER
MAX	4.6549	3.0174	-1.2591	10.5689
RATE	0.0119	0.0147	-0.0169	0.0408
LAG	-11.2803	18.2102	-46.9716	24.4111

ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

	MAX	RATE	LAG
MAX	1.0000		
RATE	-0.9587	1.0000	
LAG	0.4454	-0.6272	1.0000

(TCW/Memos/HSM-9401)